

# Susceptibility of some traditional pear cultivars of Hungarian and foreign origin to the pathogenic bacterium *Erwinia amylovora*

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**Summary:** Fire blight, a disease caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., has been causing serious damage in Hungarian pear plantations since 1996. A prospective control measure could be the use of resistant cultivars. For that purpose ten pear cultivars have been tested under laboratory and greenhouse conditions for resistance to *Erwinia amylovora* strains collected in Hungary. Six of these cultivars are Hungarian ones of unknown origin, while four are traditional old varieties. Resistant cultivars should serve as germplasm for future breeding. Inoculations were made with a mixture of different pear isolates of the bacteria collected from various growing regions of Hungary (Ea 21, 23), at a density of  $5 \times 10^8$  cells/ml. Susceptibility/resistance has been assessed on the basis of intensity of blight symptoms observed on shoots, flower parts and fruits. Cultivars were assigned to three susceptibility groups (low, moderate and high). Complete resistance was not found among the cultivars tested. The highest level of resistance was found in cultivar 'Kieffer', while the other cultivars displayed either moderate or high susceptibility to infection.

**Key words:** traditional pear cultivars, fire blight susceptibility, *in vitro* testing

## Introduction

*Erwinia amylovora* (Burrill) Winslow et al., the bacterium that causes fire blight, appeared first in 1996 on Hungarian apple (Hevesi, 1996), subsequently on pear plantations. Due to the extreme susceptibility of pear, it caused heavy losses in all growing regions and has been the most important pathogen of pear since its appearance. Effective sanitary measures so far have not been implemented and the use of antibiotics is about to be prohibited. Therefore, utilization of cultivars that display reduced susceptibility (resistance) is of pivotal importance. In this paper we have investigated traditional old pear cultivars for their resistance to fire blight, their potential for use in resistance breeding and the possibility of their maintenance in modern plantations without the danger of severe damage during epidemics.

Research has been initiated in 1999, therefore, data of 3 successive years have been processed. Experiments were performed in the *Erwinia* laboratory of the Department of Fruit Science. Since the bacterium is a quarantine pathogen, its use in field tests is prohibited. During evaluation of fire blight resistance we could rely on data reported by earlier publications (Arsenijevič & Panič, 1992, Lézec & Belouin, 1991, van der Zwet & Bell, 1990, 1995, Spotts & Mielke 1999, Sobiczewski et al, 1997, Thiboult et al, 1989). Some of the cultivars have not been tested in abroad yet, partly because of their Hungarian origin, whereas with others infection by Hungarian *Erwinia amylovora* isolates could produce a different reaction. Susceptibility/resistance of pear

cultivars has been assessed by the authors cited based on the severity of symptoms observed on shoots and flowers. However, no literature data are available on susceptibility of unripe pear fruits. Therefore, we have categorized the investigated cultivars based on susceptibility of all three plant organs (shoots, flowers, and unripe fruits).

The present paper deals primarily with fire blight resistance of traditional old pear cultivars, on the other hand, some important commercial cultivars, newly bred, allegedly resistant cultivars and Japanese pear cultivars and their hybrids are also being investigated in our laboratory.

## Material and method

In a preliminary test we have scored the virulence of different isolates of the pathogenic bacterium. Samples have been collected from ten growing sites and different host plants, and used to infect unripe fruits of two pear cultivars, 'Kaiser Alexander' and 'Conference'. Two bacterial isolates have been selected that display a similar degree of high virulence, originating from Sarkad and Nagykanizsa (East and West part of Hungary), respectively (Ea 21 and Ea 23). Test-inoculations were performed with a mixture of these isolates at a concentration of  $5 \times 10^8$  cells/ml. For cultivation King-B agar medium was used. Cultures were conserved by lyophilization.

Pear varieties tested are shown in Table 1. Plant material was obtained from the gene bank of the experimental station of the university at Szigetcsép. Besides the assortment of



Table 1 Pear cultivars examined (Mohácsy &amp; Porpáczy, 1954, Tomcsányi, 1979, Gödörné, 2000)

Cultivars	Origin	Time of harvest	Susceptibility according to the literature
Bohusné vajkörtéje	Selected by Bohus G. (Hungary)	Early August	No data
Flemish Beauty	Random seedling, Belgium (1800)	Mid of September	Susceptible
Ferenc vérbélű	unknown	End of July, early August	No data
Ilonka	Selection of Duvals in Hungary-	End of August	No data
Kieffer	<i>Pyrus serotina</i> x Vilmos USA (1863)	Early to mid of September	Less susceptible
Magyar kobak	GYDKFV gene-bank (Hungary)	Mid of September	No data
Marik kedveltje	Random seedling of Nagykanizsa (Hungary)	Early August	No data
Mosoly	GYDKFV gene-bank (Hungary)	End of July – early August	No data
Vicar of Winkfield	Random seedling France (1760)	End of September - early October	Very susceptible
Williams	Random seedling England (1770)	Mid to end of August	Medium susceptible

nine traditional old pear cultivars 'Williams' was used as control, represents a worldwide know commercial cultivar, which has been described by several authors as susceptible (Le Lezec et al., 1998, van der Zwet & Beer, 1995).

Susceptibility of the cultivars has been tested on shoots of container-grown grafts, flowers of bearing twigs and, unripe fruits collected from trees.

The applied methods were developed according to papers published previously by other investigators (van der Zwet & Keil, 1979), and us (Hevesi et al, 2000).

Shoot inoculations were performed on 30–40 cm long shoots that developed from grafts made during the winter and grown in containers, (10 shoots/cultivar) in the spring of 2001 2002 and 2003. Shoots were punctured by a needle connected to a syringe containing the bacterial suspension at the axil of the third leaf below the shoot tip. Subsequently, shoots were covered with transparent plastic bags in order to maintain the high humidity favoring bacterial multiplication. Symptoms were evaluated one week after inoculation and repeated every fourth day over a period of three weeks.

The severity of fire blight symptoms on leaves, leaf veins and shoots was rated according to a scale of 0–5, (infection index, Horsfall & Barratt, 1945).

- 0 = the inoculation (puncture) site dries, the infection does not spread further;
- 1 = shoot browning above and below the inoculation site, leaves stay healthy;
- 2 = browning of shoots more severe, it progresses up to the main rib of the leaves;
- 3 = the shoot above the puncture becomes brown and is bended;
- 4 = leaves are brown above and below the inoculation site, but there are still healthy leaves;
- 5 = the whole shoot and leaves are brown

The disease rating on shoots is expressed by the formula:  $DR_s = \sum f_i * n_i/n$  (modified from Bertrand & Gottwald, 1978).

$DR_s$  = the severity of disease on grafts;

$f_i$  = scale value (index of infection);

$n_i$  = frequency referring to the infection index;

$n$  = number of plant parts/shoot examined within the respective cultivar.

To trace the infection of flowers was compared with two different methods of inoculation. In 2001 and 2002, short and medium long branches at the beginning of flowering were cut and kept in water at room temperature under laboratory conditions. Open flowers were sprayed with bacterial suspension, while control flowers/branches were sprayed with sterile distilled water. Not only whole flowers were assayed for susceptibility but also the differences among individual flower parts were recorded, assays were performed four days after spraying. Progression of bacterial infection was monitored visually based on the extent of browning in the receptacle, calyx, petal, pistil, and stamen. Fire blight symptoms in flowers developed rapidly and petals fall down quickly, therefore in 2003 ten flowers per cultivar were put into Petri dishes, and infection was carried out by placing the bacterial suspension on the pistil through a capillary vessel. Evaluation ensued four days later. Susceptibility of flower parts was expressed on a scale of 0–3:

0 = symptomless,

1 = browning of up to 33% of the flower organ,

2 = browning of up to 66%-of the flower organ,

3 = the whole flower organ died (total browning).

The degree of disease on flower organs ( $DR_f$ ) (receptacle:  $f_r$ , calyx:  $f_c$ , petals:  $f_{pe}$ , stamen:  $f_s$ , pistil:  $f_{pi}$ ) was expressed using the same formula as used for shoots.

On unripe fruits, natural infection through superficial scars was imitated in the laboratory. For that purpose fruits of 2–2.5 cm diameter were used (six fruits/cultivar). Six punctures per fruit were made with a needle dipped into the bacterial suspension. The same procedure was performed with needles dipped into distilled water as a control. In order



to maintain the necessary humid atmosphere, inoculated fruits were placed in transparent plastic boxes. Susceptibility of cultivars was rated on basis of appearance of expanding water soaked spots around inoculation sites, whereas the appearance of dry, depressed, consistent spots with a clear cut margin was considered as a sign of resistance. The diameter of water soaked/necrotic spots and the size of mucilage drops appearing on the surface served as parameters of the degree of susceptibility. Evaluation ensued at the 4th day after inoculation according to a scale between 0–5, as follows:

- 0 = symptomless fruit;
- 1 = 1–5 mm diameter of necrotic spots,
- 2 = 6–10 mm diameter of necrotic spots,
- 3 = 11–20 mm of water soaked spots,
- 4 = 21–30 mm of water soaked spots,
- 5 = diameter of water soaked spots is more than 31 mm.

Disease severity on fruit ( $DR_{fr}$ ), as well as categorization of cultivars has been calculated by the same formula as the one used in shoot tests.

Statistical analysis was carried out with the aid of the SPSS software, by using a one variable variance analysis. Graphs were drawn with the aid of an Excel XP table-management software. Categorization of cultivars in susceptibility groups was performed by Cluster analysis.

**Results**

**Shoot-infection.** A relatively outstanding resistance has been displayed by cultivar 'Kieffer', which is in accordance with earlier literature data (Bellini & Nin, 1997). Furthermore, our research has shown that within a three-year assay period the cultivar 'Flemish Beauty' turned out to be the most susceptible (Figure 1). The statistics processed according to the SPSS software revealed three clusters of the cultivars. The first cluster (low susceptibility) is represented by a single cultivar, 'Kieffer', which is clearly distinct from the rest (Figure 2). Based on the symptoms observed on

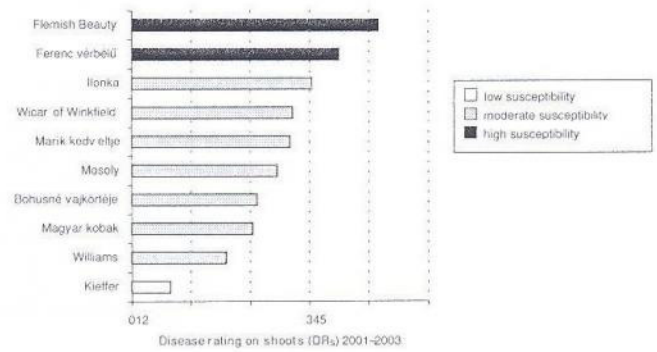


Figure 1 Susceptibility of shoots of pear cultivars to the bacterium *Erwinia amylovora* (2001–2003)

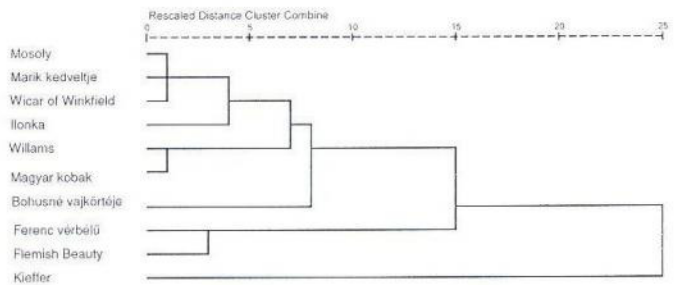


Figure 2 Dendrogram of cultivars showing fire blight disease severity according to data obtained in 2001, 2002 and 2003

shoots most of the cultivars evaluated displayed moderate susceptibility: 'Mosoly', 'Marik kedveltje', Vicar of Winkfield', 'Ilonka', 'Williams', 'Magyar kobak' and 'Bohusné vajkörtéje'. Very susceptible cultivars were 'Ferenc vérbélű' and 'Flemish Beauty'.

**Flower-infection.** In 2001 and 2002, frost damage interfered with our project, thus only a few flower-tests have been performed. Therefore, only the data of 2003 are available (Table 2). According to our opinion, susceptibility of the receptacle and the calyx of flowers are most indicative

Table 2 Susceptibility of flower parts to infection by *Erwinia amylovora* (04. 26. 2003.)

Cultivars	$DR_{pi}^f$ (pistil)	Group of homogeneity	$DR_{pe}^f$ (petals)	Group of homogeneity	$DR_{c}^f$ (calyx)	Group of homogeneity	$DR_{re}^f$ (receptacle)	Group of homogeneity	$DR_{s}^f$ (stamen)	Group of homogeneity
Flemish Beauty	0.10	C	0.00	C	0.40	C	0.30	C	0.00	C
Kieffer	2.90	B	0.00	C	0.40	C	0.20	C	0.00	C
Bohusné vajkörtéje	2.40	B	0.20	C	1.30	B	1.20	B	0.40	BC
Ilonka	3.00	A	3.00	A	1.00	BC	1.60	B	0.40	BC
Williams	3.00	A	0.60	BC	2.10	B	2.60	AB	0.80	B
Mosoly	3.00	A	1.00	B	2.38	B	2.63	AB	2.00	AB
Magyar kobak	2.80	B	1.60	B	2.60	A	2.40	AB	2.10	AB
Vicar of Winkfield	3.00	A	1.00	B	2.50	AB	3.00	A	2.20	A

$DR_{pi}^f$  = infection of pistil (calculated DRf value)  
 $DR_{pe}^f$  = infection of the petals  
 $DR_{c}^f$  = infection of the calyx

$DR_{re}^f$  = infection of the receptacle  
 $DR_{s}^f$  = infection of the stamen



because the pathogen enters into flowers through pistil. Therefore, primarily these organs have been considered in our tests. Least susceptible cultivars proved to be 'Flemish Beauty' and 'Kieffer' (Figure 3), which corresponded with literature data in the case of 'Kieffer' as well as with results obtained in shoot infections. In 'Flemish Beauty', the results of flowers contradict those of the shoot tests because the latter proved to be very susceptible. The most susceptible flowers were found in cultivars 'Magyar kobak' and 'Vicar of Winkfield' (Figure 4). 'Ilonka', 'Bohusné vajkörtéje', 'Williams' and 'Mosoly' are moderate in susceptibility of flowers.

**Fruit-infection** was rated according to two criteria, the diameter of spots around the inoculation (puncture) site and the diameter of mucilaginous drops (ooze), as described previously (materials and methods). 'Vicar of Winkfield' proved to be resistant as the spots were dark and desiccated,

which was unique among the whole assortment. (Figure 5). Most susceptible was 'Ilonka' according to both criteria, i.e. diameters of browning and of the mucilaginous drop. In addition, fruits of 'Bohusné vajkörtéje' were also very susceptible (Figure 6). 'Kieffer', the cultivar most resistant to shoot infection, proved to be moderately susceptible according to the fruit tests along with 'Williams' (Figure 7).

For the final evaluation of fire blight susceptibility of pear cultivars cumulative data of shoots, flower organs and fruits were considered, in order to carry out a homogeneity test (Tukey B test) (Table 3). The cultivar 'Kieffer' represented the less susceptible category. 'Williams', 'Marik kedveltje' and 'Mosoly körte' were moderately susceptible. 'Magyar kobak' and 'Ferenc vérbélű' could not be rated statistically because of lack of sufficient data. Therefore, the most susceptible cultivars besides 'Flemish Beauty' were



Figure 3 Flowers of cultivar 'Kieffer' 4 days after being infected (Photo: Honty Krisztina, 2003)



Figure 4 Flowers of cultivar 'Vicar of Winkfield' 4 days after being infected (Photo: Honty Krisztina, 2003)



Figure 5 Fruits of 'Vicar of Winkfield' 4 days after being infected (Photo: Honty Krisztina, 2003)

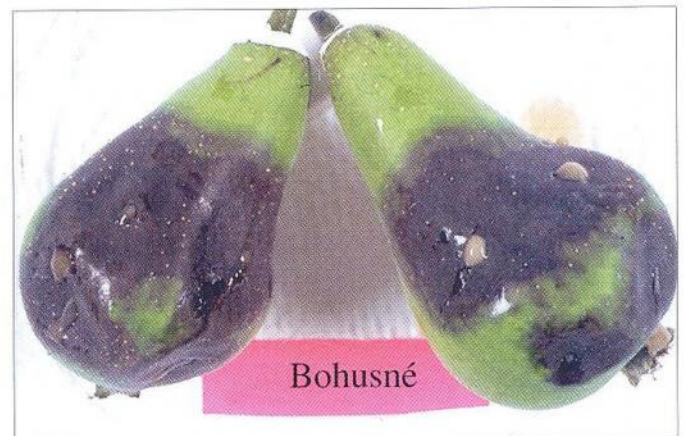


Figure 6 Fruits of 'Bohusné vajkörtéje' 4 days after being infected (Photo: Bogár László, 2001)



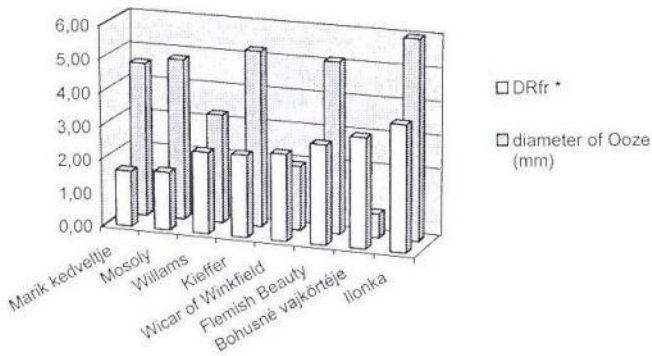


Figure 7 Susceptibility of unripe fruits to infection by *Erwinia amylovora* (2001)

Table 3 Homogeneity test of the homogeneity groups (Tukey B) (2001–2003)

Cultivars	Homogeneity groups
Kieffer	C
Williams	B
Marik kedveltje	B
Mosoly	B
Vicar of Winkfield	AB
Bohusné vajkörtéje	AB
Flemish Beauty	A
Ilonka	A

'Ilonka', 'Vicar of Winkfield' and 'Bohusné vajkörtéje'. According to our results, the cultivar 'Kieffer' deserves special attention as a potential source of resistance for future breeding programs.

## Acknowledgements

This research was supported by grants from the Hungarian Scientific Research Fund (T 035120, T 043279).

## References

Arsenijević, M. & Panič, M. (1992): First appearance of fire blight, caused by *Erwinia amylovora*, on quince and pear in Yugoslavia. *Plant Disease*, 76 (12): 1283.

Bellini, E. & Nin, S. (1997): Il miglioramento genetico del pero nel mondo. *Rivista di Frutticoltura*, 3:19–30.

Bertrand, P. F. & Gottwald, T. R. (1978): Evaluating fungicides for pear disease control. in: Zehr E. I. (Ed.) *Methods for Evaluating Plant Fungicides, Nematicides and Bactericides*. St. Paul Minnesota, 179–181.

Göndör J-né. (ed.) (2000): *Körte*. Mezőgazda kiadó, Bp.

Hevesi M. (1996): Az *Erwinia amylovora* (Burrill) Winslow et al. hazai megjelenése almán. *Növényvédelem*, 32 (5): 225–228.

Hevesi M., Papp J., Jámor-Benczúr E., Kaszáné Csizmár K., Pozsgai I., Gazdag Gy. & Balla I. (2000): Testing the virulence of some Hungarian *Erwinia amylovora* strains on in vitro cultured apple rootstocks. *Horticultural Science*, 6 (4): 52–55.

Horsfall, J. G. & Barratt, R. W. (1945): An improved grading system for measuring plant disease. *Phytopathology*, 35: 655.

Le Lezec, M. & Belouin, A. (1991): L'Amélioration du poirier pour la résistance au feu bacterien. *L'Arboriculture Fruitière*, 440: 29–37.

Le Lezec, M., Laurens, F., Michelesi, J. C. & Lecomte, P. (1998): Suscettibilità varietale del melo e del pero al „colpo di fuoco batterico“. *Rivista di Frutticoltura*, 3, (9): 9–14.

Sobiczewski, P., Deckers, T. & Pulawska, J. (1997): Fire Blight (*Erwinia amylovora*), Some Aspects of Epidemiology and Control. *Res. Inst. of Pomology and Floriculture*. Poland., 43–46.

Spotts, R. A. & Mielke, E. A. (1999): Resistance of pear cultivars in Oregon to natural fire blight infection. *Fruit Varieties Journal*, 53(2):110–115.

Thibault, B., Belouin, A. & Lecomte, P. (1989): Sensibilité varietale du poirier au feu bacterien. *L'Arboriculture Fruitière*, 421: 29–34.

Tomesányi P. (ed.) (1979): *Gyümölcsfajtáink, gyakorlati pomológia*. Mezőgazdasági Kiadó, Bp.

van der Zwet, T. & Bell, R. L. (1990): Fire blight susceptibility in *Pyrus* germplasm from Eastern Europe. *HortScience*, 25(5): 566–568.

van der Zwet, T. & Bell, R. L. (1995): Response of Central European *Pyrus* germplasm to natural fire blight infection and artificial inoculation. *HortScience*, 30 (6): 1287–1291.

van der Zwet, T. & Keil, H. (1979): *Fire Blight. A Bacterial Disease of Rosaceous Plants*. U.S. Government Printing Office, Washington, 510.

van der Zwet, T. & Beer, S. W. (1995): *Fire Blight – Its Nature, Prevention and Control*. USDA Bulletin 631: 1–97.